PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
69308		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/06969	07/04/1998	08/04/1997
International Patent Classification (IPC) or national classification and IPC		
B01D65/06		
Applicant		
PALL CORPORATION et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/11/1998	05.07.99
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Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US98/06969

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-8,10,14,17, 19-28	as originally filed			
9,11-13,15,16, 18	as received on	04/02/1999	with letter of	01/02/1999

Claims, No.:

9-35	as originally filed			
1-8	as received on	04/02/1999	with letter of	01/02/1999

Drawings, sheets:

1/4-4/4	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-35
	No:	Claims
Inventive step (IS)	Yes:	Claims 1-35
	No:	Claims
Industrial applicability (IA)	Yes:	Claims 1-35
	No:	Claims

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

V

1.1 The present invention pertains to a method for cleaning a porous membrane which has been used for filtering beer, wherein the membrane is treated with an enzyme selected from the group consisting of cellulase, amylases, and combinations thereof, in the absence of protease and glucanase.
D1:WO-A-9 623 579 discloses a method for cleaning membrane filters by using an enzyme-containing solution, which comprises, among other enzymes, e.g. a combination of glucanases, xylanases and cellulase, i.e. the defined enzyme combination of the present claim 1 is not disclosed in D1.
Since the treatment with the enzyme combination as defined in claim 1 leads to an better and more gently cleaning operation, it has to be considered to contain an inventive step over the disclosure of D1. Thus, the subject-matter of the amended claim 1 is considered to meet the requirements of Article 33.3 PCT (see 2. below).

1.2 The dependent claims 2 to 35 refer to specific embodiments of the invention and do thus also appear to satisfy the requirements of Articles 33.2 and 33.3 PCT (see 2.1 below).

VIII

2.1 The present independent claims 4 and 29 contain all of the features of the present claim 1 and are thus specific embodiments of the invention as defined in claim 1. Thus, in order to fulfil the requirements of Article 6 PCT, these claims should accordingly be drafted as dependent claims on said claim 1. The common features of claim 4 and 29 to those of claim 1 should then be omitted from these claims.
Further, the independent claims 1,4 and 29 do not satisfy the requirements of Rule 13.1 PCT in the light of the disclosure of D1.
Even further, it appears to be more appropriate that the above mentioned claims should be directed to "A method for filtering beer" instead of "A method for producing beer".

2.2 The wording "consisting of....cellulase, amylases and combinations thereof" of claim 1 should, of course, be interpreted to mean that no other enzymes than the defined ones should be used, i.e. the conventional definition of the wording "consisting of". The counterparts of the description should then be adapted correspondingly (see

page 5, lines 23 to 27; Rule 5.1(a)(i)-(vi) PCT). The present claim 2 should thus be deleted, since it implies that other enzymes also may be present (Art.6 PCT).

method of the present invention has filtration halted at a point when the filter's zeta potential has decreased to a maximum of 20% of the value it exhibited in its unused state, or when clogging does not exceed 80%.

Another refinement of the process will use a porous membrane of polyamide, with filtration halted when the zeta potential exceeds -5 mV as measured at a pH of 4.2.

The beer preferably will undergo pre-filtration before filtration proper, i.e., filtration through the porous membrane. Diatomateous (or infusorial) earth, also known as diatomite, is almost exclusively used for pre-filtration. A combination of diatomateous earth and deep-bed filtration also is feasible.

The present invention can be used in any suitable beer production system. Preferably, the present invention is used in connection with the cluster filter system as described in U.S. Patents 5,417,101 and 5,594,161.

The present invention also relates to a filtration unit for filtering beer, with a feeder line for the filtration-bound beer, a porous membrane, and a run-off line for the filtered beer. It is characterized by a module in the form of a meter cell, functioning as bypass, and featuring a porous membrane and means, e.g., electrodes, for monitoring the streaming potential and/or zeta potential of the meter cell's membrane filter through which beer flows.

The present invention also deals with a filtration unit for filtering beer, with the unit featuring a feeder line for filtration-bound beer, a porous membrane, and a run-off line for filtered beer. In divergence from the foregoing paragraph, the filtration unit is characterized by means, e.g., electrodes, being attached to the porous membrane for monitoring or reading the streaming potential and/or zeta potential as the beer flows through the porous membrane. In this variation, the zeta potential is not measured via the meter cell assigned as

By this procedure, the cleaning process can be evaluated and/or optimized for it's efficiency:

4. The aging of a porous membrane for reasons of repeated use can be tracked, providing a handy estimate as to its remaining service life expectancy.

5. By measuring zeta potential, filter material and shunting materials (e.g., diatomite, bentonite, perlite, polyvinyl pyrrolidone) can be tested for suitability in beer filtration by assessing the interaction between clogging substances of liquid systems and filter material and/or shunting means for filters.

6. The service life of a porous membrane can be estimated by way of measuring zeta potential, whereunder a specific membrane load (hl/m^2) is recorded up to the point when clogging sets in.

The artisan is aware that most suitable for the process are porous membranes with a zeta potential exhibiting pronounced change in relation to the degree of clogging. Verification of these parameters is easy enough by employing the aforementioned simple test method.

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

This example illustrates the effectiveness of the present inventive method to produce beer. In particular, this example demonstrates that cellulases and amylases can be used to satisfactorily clean a porous membrane clogged in the course of beer filtration such that the porous membrane can be reused in continued beer filtration.

A porous membrane made of nylon-6,6 (NB type, commercially available from Pall Filtrationstechnik GmbH, Germany) was used as a filter. Such a filter is frequently used in the state of the art for the cold-filtration of beer.

The so-called membrane filter test according to Esser (Monatszeitschrift für Brauerei (Monthly Magazine for Breweries), 25th year, No. 6, pages 145-151, 1972) was used to determine the filtering performance of the filter. This test is reliable for checking measures for improving filterability.

To determine the filtering efficiency of a new, i.e., unused, porous membrane, a pressure filtration apparatus (SM 16526 type, 200 ml capacity; commercially available from Sartorius GmbH, Goettingen, Germany) was used for a polyamide nylon-6,6 porous membrane having a 47 mm diameter and a 0.2 µm pore size.

Beer cooled down to 0 °C was forced through the porous membrane under isobaric conditions (1 bar), and the amount of filtrate was weighed every 10 seconds. The test was stopped after 200 g of filtrate were obtained. The result is shown as a graph in the diagram of Figure 1. Figure 1 shows that, under the conditions indicated above, the 200 g of filtrate were obtained with the unused filter after approximately 210 seconds.

Under identical conditions, the filtering performance of a clogged, i.e., used, porous membrane was tested. The result is given in Figure 2 which shows that even in 720 seconds only approximately 60 g of filtrate were obtained.

The clogged porous membrane was cleaned in accordance with a prior art method, wherein the membrane was first cleaned enzymatically and then chemically, as described below.

For enzymatic cleaning, the clogged membrane was treated for 1 hour with a 1% aqueous solution of a mixture of β-glucanases and xylanases (P3-Ultrasil 65; commercially available from Henkel) with a pH of 5 (adjusted with a 0.05% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; commercially available from Henkel)) at a temperature of 50 °C. This treatment was subsequently carried out one more time.

The membrane was then treated for 3 hours with a 0.5% aqueous solution of a mixture of surfactants, glucanases, and proteases (P3-Ultrasil 62; commercially available from Henkel) with a pH of 9-9.5 (set with a 0.15% aqueous solution of a mixture of surfactants and an alkaline component (P3-Ultrasil 91; commercially available from Henkel)) at a temperature of 50 °C and subsequently rinsed with warm water (50 °C).

For chemical cleaning, the membrane thereafter was treated for 30 minutes with a 1% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; commercially available from Henkel) at 60 °C, and then rinsed with fresh water. The membrane was subsequently treated for 30 minutes with an aqueous solution containing 1% of a mixture of surfactants and an alkaline component (P3-Ultrasil 91; commercially available from Henkel) and 1% of a mixture of surfactants and an oxygen donor (P3-Ultrasil 05; commercially available from Henkel) at a temperature of 60 °C and then rinsed with fresh water. The membrane was then treated once more for 30 minutes with a 0.5% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; commercially available from Henkel) and subsequently rinsed with fresh water until the rinse water reached the electrical conductivity of fresh water.

The filtering performance of this cleaned porous membrane was then tested again under the conditions indicated above. The result is shown in Figure 3. Figure 3 shows that the filtering performance has improved somewhat as the 200 g of filtrate were obtained after approximately 600 seconds.

A similarly clogged membrane whose filtering efficiency is shown in Figure 2 was cleaned in accordance with the method according to the present invention. The membrane was treated for 30 minutes with an aqueous solution of C_1- and C_x-cellulases, the solution having a pH value of 4.7, at a temperature of 45 °C. The membrane was then treated with the same solution, but at a pH value of 5.0 and a

was determined. Reference numeral 1 designates the measuring cell in which the porous membrane 2 is clamped without warping in filter holders 3 and 4 made of polytetrafluoroethylene. The filter holders 3 and 4 are the end pieces of two pistons 5 and 6, respectively, which are mounted for displacement in the cylindrical part 7 of the measuring cell 1.

The end pieces 3 and 4 of the pistons 5 and 6, respectively, have fine bores 10 and 11 for the fluid which is to be filtered and press the perforated electrodes 8 and 9 against the porous membrane 2. The electrodes 8 and 9 are connected to the two electric terminals 12 and 13 extending inside the pistons 5 and 6 so the streaming potential built up as fluid flows through the membrane 2 can be measured. Silver electrodes or silver chloride electrodes which exhibit a low polarization during passage of current are preferred for the electrodes. The pistons 6 and 7 are mounted in the seals 14 and 15, respectively, such that, on the one hand, they are displaceable, and, on the other hand, they do not allow any fluid to leak from the measuring cell.

The fluid to be filtered flows through the supply line 16 into the cylindrical part 7 of the measuring cell 1, through the fine bores 10 of the piston 6, through the electrode 8, with an electric potential being built up, and through the porous membrane 2. The filtered fluid flows through the electrode 9, with a potential likewise being built up, passes through the fine bores 11 of the piston and leaves the measuring cell through the discharge line 17.

To determine the zeta potential from the measured streaming potential, measurement (not illustrated) of the differential pressure in the measuring cell between supply line 16 and discharge line 17, the conductivity and also the pH value is necessary. The zeta potential is calculated from these measured quantities as follows:

$$\text{zeta potential} = \frac{U}{\Delta p} \cdot \frac{LF.\eta}{\varepsilon.\varepsilon_0}$$

where U is the streaming potential, Δp the pressure difference, LF the conductivity, η the viscosity, and $\varepsilon\varepsilon_0$ the dielectric constant.

The change in the zeta potential of the membrane filter as clogging progresses is shown in Figure 6. This figure is a diagram in which the zeta potential in millivolts is plotted as ordinate, and the pH value at which the zeta potential was determined as abscissa. The pH value of the electrolyte solution (0.001 N aqueous KCl solution) was set with 0.1 N HCl or with 0.1 N NaOH. The specified pressure difference was 350 mbar.

The diagram was obtained by first determining with the measuring cell described above the zeta potentials of a new, i.e., unused porous membrane made of polyamide (NB type, commercially available from Pall Filtrationstechnik GmbH, 6072 Dreieich 1, Germany) at various pH values.

The results relating to the unused porous membrane are plotted as curve "a". It is evident that the unused porous membrane has a zeta potential of approximately -18 mV with an alkaline pH, and that the zeta potential increases with decreasing pH and finally reaches zero value at a pH of approximately 3.

Curve "b" shows the dependence of the zeta potential on the pH value of the porous membrane under identical measuring conditions, as stated above, but after use thereof for filtering beer and, therefore, with partial clogging. As is apparent, the zeta potential is raised somewhat by the partial clogging and only reaches a value of approximately -15 mV at pH values of approximately 7.

Curve "c" was plotted for the same porous membrane in the nearly fully clogged state. It is evident that the zeta potential now changes only slightly with the pH value, and even in the alkaline range does not fall below approximately -2 mV.

equal to the amount of porous membrane surface per cm^2 in the filtration chamber 18.

The severe change in filter test membrane 2 (Figure 5) zeta potential inside meter cell 1 during filtration allows an assessment of the state of the filter candles 19 in filtration chamber 18.

EXAMPLE 3

This example illustrates the effectiveness of cellulase derived from *Aspergillus niger* in enzymatically degrading soluble and crystalline cellulose substrates.

Cellulase derived from *Aspergillus niger* was obtained from Fluka (item numbers 22178). The enzyme was evaluated with respect to two different celluloses: soluble carboxymethylcellulose (CMC, available from Aldrich as item number 41927-3) and crystalline cellulose (Avicel, available from FMC as item number PH-105).

The test methodology involved the preparation of an incubation solution of (i) 18 ml CMC (1%) or Avicel (1%), (ii) 5 ml sodium acetate buffer (50 mM, pH 4.8), and (iii) 5 ml of a solution of the enzyme in sodium acetate buffer (50 mM, pH 4.8) at 30 °C. A test solution then was prepared by mixing 1.4 ml of the incubation solution with 0.1 ml glucose solution (0.15%) and 1.5 ml 3,5-dinitrosalicylic acid (DNS) reagent (available from Sigma as item number D-0550). The test solution was boiled for 15 minutes. The total µmol glucose equivalents/mg enzyme as a function of time (min) was determined spectroscopically (575 nm), using two parallel samples, in accordance with the procedure described in Miller, *Anal. Chem.*, *31*, 426-28 (1959), using a straight line calibration with a glucose standard. Protein amounts were determined in accordance with the procedure described in Bradford, *Anal. Biochem.*, *72*, 248-64 (1976), using a bovine serum albumin (BSA) standard.

The enzymatic degradation of cellulose results in the production of glucose, and, therefore, the measurement of

WHAT IS CLAIMED IS:

1. A method for producing beer comprising filtering beer through a porous membrane until such time that said porous membrane is in need of cleaning, contacting said porous membrane with an enzyme selected from the group consisting of cellulases, amylases, and combinations thereof in the absence of a protease or a glucanase to clean said porous membrane, and then reusing said porous membrane to continue filtering beer.

2. The method of claim 1, wherein said porous membrane is not contacted with an enzyme other than said cellulase or said amylase.

3. The method of claim 1 or 2, wherein said porous membrane is contacted with said cellulase.

4. A method for producing beer comprising filtering beer through a porous membrane until such time that said porous membrane is in need of cleaning, contacting said porous membrane with a cellulase having a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.1 to clean said porous membrane, and then reusing said porous membrane to continue filtering beer.

5. The method of claim 3 or 4, wherein said porous membrane is contacted with said cellulase and is not contacted with any other enzyme.

6. The method of any of claims 1-3 or 5, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.1.

7. The method of claim 6, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.3.

8. The method of claim 7, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.4.

ENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
69308		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 98/ 06969	07/04/1998	08/04/1997
Applicant		
PALL CORPORATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. 4
   - [X] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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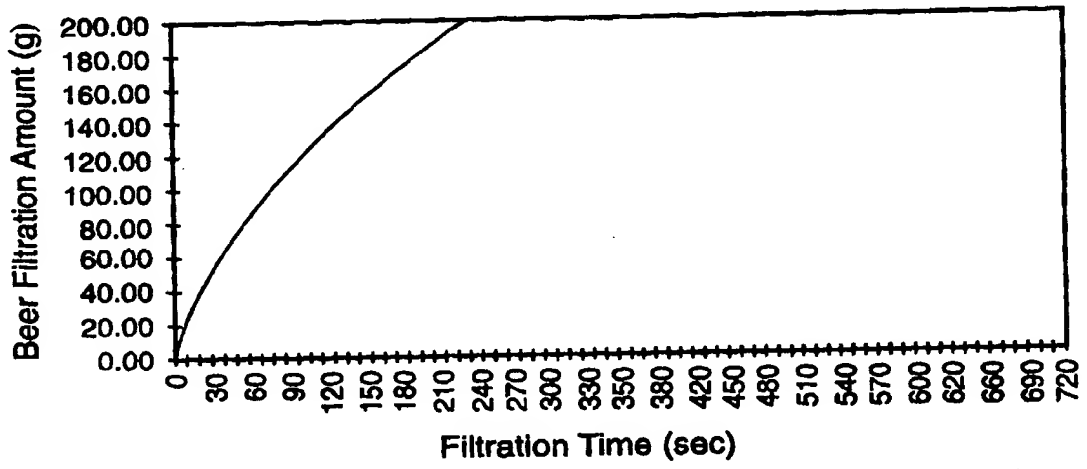
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR PRODUCING BEER



(57) Abstract

The present invention provides a method for producing beer comprising filtering beer through a porous membrane until such time that the porous membrane is in need of cleaning, contacting the porous membrane with an enzyme selected from the group consisting of cellulases, amylases, and combinations thereof, particularly a cellulase having a crystalline: soluble cellulose activity ratio at 60 minutes of at least about 0.1, to clean the porous membrane, and then reusing the porous membrane to continue filtering beer. The present invention further provides a method for producing beer comprising filtering beer through a porous membrane that progressively clogs during filtration, monitoring the streaming or zeta potential of the porous membrane as a measure of the extent of clogging of the porous membrane, halting filtration of the beer through the porous membrane before the porous membrane becomes fully clogged as determined by the streaming or zeta potential of the porous membrane, cleaning the porous membrane, and then reusing the porous membrane to continue filtering beer.

FOR THE PURPOSES OF INFORMATION ONLY

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MN	Mongolia
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MW	Malawi
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NL	Netherlands
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UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

WHAT IS CLAIMED IS:

1. A method for producing beer comprising filtering beer through a porous membrane until such time that said porous membrane is in need of cleaning, contacting said porous membrane with an enzyme selected from the group consisting of cellulases, amylases, and combinations thereof in the absence of a protease or a glucanase to clean said porous membrane, and then reusing said porous membrane to continue filtering beer.

2. The method of claim 1, wherein said porous membrane is not contacted with an enzyme other than said cellulase or an amylase.

3. The method of any of claim 1 or 2, wherein said porous membrane is contacted with said cellulase.

4. A method for producing beer comprising filtering beer through a porous membrane until such time that said porous membrane is in need of cleaning, contacting said porous membrane with a cellulase having a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.1 to clean said porous membrane, and then reusing said porous membrane to continue filtering beer.

5. The method of claim 3 or 4, wherein said porous membrane is contacted with said cellulase and is not contacted with any other enzyme.

6. The method of any of claims 1-5, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.1.

7. The method of claim 6, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.3.

8. The method of claim 7, wherein said cellulase has a crystalline:soluble cellulose activity ratio at 60 minutes of at least about 0.4.

equal to the amount of porous membrane surface per cm^2 in the filtration chamber 18.

The severe change in filter test part 2 (Figure 5) zeta potential inside meter cell 1 during filtration allows an assessment of the state of the filter candles 19 in filtration chamber 18.

EXAMPLE 3

This example illustrates the effectiveness of cellulase derived from *Aspergillus niger* in enzymatically degrading soluble and crystalline cellulose substrates.

Cellulase derived from *Aspergillus niger* was obtained from Fluka (item numbers 22178). The enzyme was evaluated with respect to two different celluloses: soluble carboxymethylcellulose (CMC, available from Aldrich as item number 41927-3) and crystalline cellulose (Avicel, available from FMC as item number PH-105).

The test methodology involved the preparation of an incubation solution of (i) 18 ml CMC (1%) or Avicel (1%), (ii) 5 ml sodium acetate buffer (50 mM, pH 4.8), and (iii) 5 ml of a solution of the enzyme in sodium acetate buffer (50 mM, pH 4.8) at 30 °C. A test solution then was prepared by mixing 1.4 ml of the incubation solution with 0.1 ml glucose solution (0.15%) and 1.5 ml 3,5-dinitrosalicylic acid (DNS) reagent (available from Sigma as item number D-0550). The test solution was boiled for 15 minutes. The total µmol glucose equivalents/mg enzyme as a function of time (min) was determined spectroscopically (575 nm), using two parallel samples, in accordance with the procedure described in Miller, *Anal. Chem.*, *31*, 426-28 (1959), using a straight line calibration with a glucose standard. Protein amounts were determined in accordance with the procedure described in Bradford, *Anal. Biochem.*, *72*, 248-64 (1976), using a bovine serum albumin (BSA) standard.

The enzymatic degradation of cellulose results in the production of glucose, and, therefore, the measurement of

$$\text{zeta potential} = \frac{U}{\Delta p} \cdot \frac{LF.\eta}{\varepsilon.\varepsilon_o}$$

where U is the streaming potential, Δp the pressure difference, LF the conductivity, η the viscosity, and $\varepsilon\varepsilon_o$ the dielectric constant.

The change in the zeta potential of the membrane filter as clogging progresses is shown in Figure 6. This figure is a diagram in which the zeta potential in millivolts is plotted as ordinate, and the pH value at which the zeta potential was determined as abscissa. The pH value of the electrolyte solution (0.001 N aqueous KCl solution) was set with 0.1 N HCl or with 0.1 N NaOH. The specified pressure difference was 350 mbar.

The diagram was obtained by first determining with the measuring cell described above the zeta potentials of a new, i.e., unused porous membrane made of polyamide (NB type, manufacturer: Pall Filtrationstechnik GmbH, 6072 Dreieich 1, Germany) at various pH values.

The results relating to the unused porous membrane are plotted as curve "a". It is evident that the unused porous membrane has a zeta potential of approximately -18 mV with an alkaline pH, and that the zeta potential increases with decreasing pH and finally reaches zero value at a pH of approximately 3.

Curve "b" shows the dependence of the zeta potential on the pH value of the porous membrane under identical measuring conditions, as stated above, but after use thereof for filtering beer and, therefore, with partial clogging. As is apparent, the zeta potential is raised somewhat by the partial clogging and only reaches a value of approximately -15 mV at pH values of approximately 7.

Curve "c" was plotted for the same porous membrane in the nearly fully clogged state. It is evident that the zeta potential now changes only slightly with the pH value, and even in the alkaline range does not fall below approximately -2 mV.

was determined. Reference numeral 1 designates the measuring cell in which the porous membrane 2 is clamped without warping in filter holders 3 and 4 made of polytetrafluoroethylene. The filter holders 3 and 4 are the end pieces of two pistons 5 and 6, respectively, which are mounted for displacement in the cylindrical part 7 of the measuring cell 1.

The end pieces 3 and 4 of the pistons 5 and 6, respectively, have fine bores 10 and 11 for the fluid which is to be filtered and press the perforated electrodes 8 and 9 against the porous membrane 2. The electrodes 8 and 9 are connected to the two electric terminals 12 and 13 extending inside the pistons 5 and 6 so the streaming potential built up as fluid flows through the membrane 2 can be measured. Silver electrodes or silver chloride electrodes which exhibit a low polarization during passage of current are preferred for the electrodes. The pistons 6 and 7 are mounted in the seals 14 and 15, respectively, such that, on the one hand, they are displaceable, and, on the other hand, they do not allow any fluid to leak from the measuring cell.

The fluid to be filtered flows through the supply line 16 into the cylindrical part 7 of the measuring cell 1, through the fine bores 10 of the piston 6, through the electrode 8, with an electric potential being built up, and through the porous membrane 2. The filtered fluid flows through the electrode 9, with a potential likewise being built up, passes through the fine bores 11 of the piston and leaves the measuring cell through the discharge line 17.

To determine the zeta potential from the measured streaming potential, measurement (not illustrated) of the differential pressure in the measuring cell between supply line 16 and discharge line 17, the conductivity, and, expediently, also the pH value is necessary. The zeta potential is calculated from these measured quantities as follows:

The membrane was then treated for 3 hours with a 0.5% aqueous solution of a mixture of surfactants, glucanases, and proteases (P3-Ultrasil 62; manufacturer: Henkel) with a pH of 9-9.5 (set with a 0.15% aqueous solution of a mixture of surfactants and an alkaline component (P3-Ultrasil 91; manufacturer: Henkel)) at a temperature of 50 °C and subsequently rinsed with warm water (50 °C).

For chemical cleaning, the membrane was treated for 30 minutes with a 1% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; manufacturer: Henkel) at 60 °C, and then rinsed with fresh water. The membrane was subsequently treated for 30 minutes with an aqueous solution containing 1% of a mixture of surfactants and an alkaline component (P3-Ultrasil 91; manufacturer: Henkel) and 1% of a mixture of surfactants and an oxygen donor (P3-Ultrasil 05; manufacturer: Henkel) at a temperature of 60 °C and then rinsed with fresh water. The membrane was then treated once more for 30 minutes with a 0.5% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; manufacturer: Henkel) and subsequently rinsed with fresh water until the rinse water reached the electrical conductivity of fresh water.

The filtering performance of this cleaned porous membrane was then tested again under the conditions indicated above. The result is shown in Figure 3. Figure 3 shows that the filtering performance has improved somewhat as the 200 g of filtrate were obtained after approximately 600 seconds.

The same clogged membrane whose filtering efficiency is shown in Figure 2 was cleaned in accordance with the method according to the present invention. The membrane was treated for 30 minutes with an aqueous solution of C_1- and C_x-cellulases, the solution having a pH value of 4.7, at a temperature of 45 °C. The membrane was then treated with the same solution, but at a pH value of 5.0 and a

The so-called membrane filter test according to Esser (Monatszeitschrift für Brauerei (Monthly Magazine for Breweries), 25th year, No. 6, pages 145-151, 1972) was used to determine the filtering performance of the filter. This test is reliable for checking measures for improving filterability.

To determine the filtering efficiency of a new, i.e., unused, porous membrane, a pressure filtration apparatus (SM 16526 type, 200 ml capacity; manufacturer: Sartorius GmbH, Goettingen, Germany) was used for a polyamide nylon-6,6 porous membrane having a 47 mm diameter and a 0.2 µm pore size.

Beer cooled down to 0 °C was forced through the porous membrane under isobaric conditions (1 bar), and the amount of filtrate was weighed every 10 seconds. The test was stopped after 200 g of filtrate were obtained. The result is shown as a graph in the diagram of Figure 1. Figure 1 shows that, under the conditions indicated above, the 200 g of filtrate were obtained with the unused filter after approximately 210 seconds.

Under identical conditions, the filtering performance of a clogged, i.e., used, porous membrane was tested. The result is given in Figure 2 which shows that even in 720 seconds only approximately 60 g of filtrate were obtained.

The clogged porous membrane was cleaned in accordance with a prior art method, wherein the membrane was first cleaned enzymatically and then chemically, as described below.

For enzymatic cleaning, the clogged membrane was treated for 1 hour with a 1% aqueous solution of a mixture of β-glucanases and xylanases (P3-Ultrasil 65; manufacturer: Henkel) with a pH of 5 (set with a 0.05% aqueous solution of a mixture of surfactants and an acidic component (P3-Ultrasil 75; manufacturer: Henkel)) at a temperature of 50 °C. This treatment was subsequently carried out one more time.

By this procedure, the cleaning process can be evaluated and/or optimized for it's efficiency:

4. The aging of a porous membrane for reasons of repeated use can be tracked, providing a handy estimate as to its remaining service life expectancy.

5. By measuring zeta potential, filter material and shunting materials (e.g., diatomite, bentonite, perlite, polyvinyl pyrrolidone) can be tested for suitability in beer filtration by assessing the interaction between clogging substances of liquid systems and filter material and/or shunting means for filters.

6. The service life of a porous membrane can be estimated by way of measuring zeta potential, whereunder a specific membrane load (hl/m^2) is recorded up to the point when clogging sets in.

The artisan is aware that most suitable for the process are porous membranes with a zeta potential exhibiting pronounced change in relation to the degree of clogging. Verification of these parameters is easy enough by employing the aforementioned simple test method.

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

This example illustrates the effectiveness of the present inventive method to produce beer. In particular, this example demonstrates that cellulases and amylases can be used to satisfactorily clean a porous membrane clogged in the course of beer filtration such that the porous membrane can be reused in continued beer filtration.

A porous membrane made of nylon-6,6 (NB type, manufacturer: Pall Filtrationstechnik GmbH, Germany) was used as a filter. Such a filter is frequently used in the state of the art for the cold-filtration of beer.

method of the present invention has filtration halted at a point when the filter's zeta potential has decreased to a maximum of 20% of the value it exhibited in its unused state, or when clogging does not exceed 80%.

Another refinement of the process will use a porous membrane of polyamide, with filtration halted when the zeta potential exceeds -5 mV as measured at a pH of 4.2.

The beer preferably will undergo pre-filtration before filtration proper, i.e., filtration through the porous membrane. Diatomateous (or infusorial) earth, also known as diatomite, is almost exclusively used for pre-filtration. A combination of diatomateous earth and deep-bed filtration also is feasible.

The present invention can be used in any suitable beer production system. Preferably, the present invention is used in connection with the cluster filter system as described in U.S. Patents 5,417,101 and 5,594,161.

The present invention also relates to a filtration unit for filtering beer, with a feeder line for the filtration-bound beer, a porous membrane, and a run-off line for the filtered beer. It is signified by a module in the form of a meter cell, functioning as bypass, and featuring a porous membrane and means, e.g., electrodes, for monitoring the streaming potential and/or zeta potential of the meter cell's membrane filter through which beer flows.

The present invention also deals with a filtration unit for filtering beer, with the unit featuring a feeder line for filtration-bound beer, a porous membrane, and a run-off line for filtered beer. In divergence from the foregoing paragraph, the filtration unit is characterized by means, e.g., electrodes, being attached to the porous membrane for monitoring or reading the streaming potential and/or zeta potential as the beer flows through the porous membrane. In this variation, the zeta potential is not measured via the meter cell assigned as

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